Effects of Processing on the Oxidative Stability of Docosahexaenoic Acid Oil

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Introduction

Omega-3 (n-3) fatty acids first gained attention when a group of nutrition experts began studying the dietary habits of Greenland Eskimos in the early 1970's (Pique 1986, Heller and others 2006(b)). The team wanted to study the effects of the Eskimos' high fat, high protein, low fiber, and low Vitamin C diet on the health of the Eskimos. They expected to find adverse health effects and high rates of heart disease, but instead they were surprised to observe remarkable general health and resistance not only to cardiovascular disease, but cancer as well (Pique 1986). In fact, the Eskimo group had a 5.3% mortality rate from heart disease, compared to approximately 40% for the U.S. population at that time (Dyerberg 1981). It was also observed that the Eskimo population had almost no cases of breast cancer. After determining that the differences were not due to genetics and exercise, the team of scientists began focusing on the Eskimos' marine animal diet, which included oily fish, whale and seal blubber (an estimated ¼ pound of fat per day). Blood analysis showed that the Eskimo population had about sixteen times the amount of eicosapentaenoic acid and approximately four times the amount of docosahexaenoic acid (Pique 1986).

Eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6, n-3) are both long-chain polyunsaturated n-3 fatty acids derived from fish and fish oils which have been reported to protect against a wide range of diseases, including: atherosclerosis, myocardial infarction, cancer, autism, and sudden death (Lee and others 2003, Mori 2004). Along with other n-3 fatty acids, the effects of EPA and DHA have been widely

reported as being beneficial to reducing inflammation, lowering blood pressure, raising low-density lipoprotein (LDL) cholesterol, and decreasing plasma triglyceride (TG) (Kasim-Karakas 2001, Mori 2004, Heller and others 2006(b)). EPA and DHA are essential for development throughout life, including for normal growth of the central nervous system; a high level of DHA is found in phospholipids of the brain and retina, suggesting an important role in optical development (Innis 1991, Lee and others 2003, Maki and others 2003).

DHA is considered essential because it needs to be ingested in the diet. A small amount can be formed inside the body from alpha-linolenic acid; however the conversion efficiency is lower than 5%. This efficiency also decreases with the intake of linoleic acid (18:2 n-6) (Borneo and others 2007). The biochemical synthesis of DHA from alpha-linolenic acid is found in significant amounts only in chloroplasts such as algae and plankton, which are the main essential fatty acid source of marine animals. DHA constitutes approximately 8-20% by weight of fish oil (Watkins and German 2008). The EPA and DHA content of several different fish can be seen in Table 1 (Heller and others 2006). The structure of DHA can be seen in Figure 1.

Table 1: EPA and DHA content of various fish

Fish	EPA (g/100g)	DHA (g/100g)
Mackerel, king	1.0	1.2
Herring, Pacific	1.0	0.7
Anchovy	0.5	0.9
Salmon, Chinook	0.8	0.6
Tuna, albacore	0.3	1.0
Salmon, pink	0.4	0.6
Halibut, Greenland	0.5	0.4

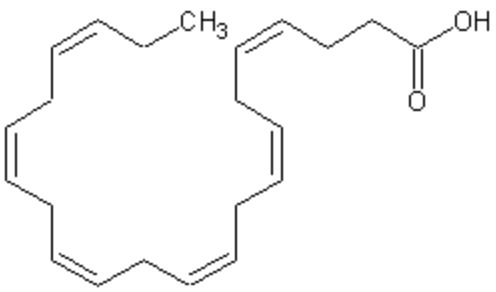

Figure 1: Structure of DHA

Historically, the intakes of omega-3, omega-6, and saturated fatty acids have been approximately equal. Western diets, however, have shifted to emphasize industrially produced vegetable oils (mostly omega-6) and animal fats (heavy in saturated fat), leaving a deficiency in omega-3 fatty acids from marine life (Heller and others 2006). A 30 g serving intake of fish per day was associated with a 50% reduction in death by heart disease over a 20-year period (Kromhout and others 1985, Lee and others 2003). Currently the American Heart Association's recommendation is at least 2 servings of fish per week, especially those high in EPA and DHA (Harris and Appel 2002).

The food industry has taken note of the increased demand for omega-3 enriched products, and the market has been rapidly growing to include more. Recently products such as enriched infant formula, margarine spreads, drink mixes, peanut butter, baked goods, and cereals have emerged. Some complications must be overcome in order to make desirable omega-3 containing products. The major concern is the deleterious effects of lipid oxidation, which increases as the number of double bonds in a fatty acid increases. Lipid oxidation causes off-flavors and aromas, which make the product unacceptable to a consumer. Since EPA and DHA are more susceptible to oxidation than vegetable oils or certainly saturated fatty acids, shelf life of enriched products is a

concern. The flavor and aroma of fish oil is also considered to be negative in many products, limiting the amount of DHA that can be added to a product. It may be possible to optimize the processing steps required for DHA oil production to improve the oxidative stability of the oil.

Processing steps involved in the production of algal DHA oil include refining, bleaching, winterizing, and deodorizing. These steps are all standard in the oil industry and are used in a variety of unsaturated oils such as soybean and canola (Johnson 2008). Each step is performed for specific purposes in order to produce oil that is acceptable to consumers for use as is, or for addition into other products. Certain prooxidant and volatile compounds are removed during these steps, along with some antioxidant compounds. Tocopherols, phospholipids, metals, chlorophylls, and peroxides are all targeted for removal during the oil production process.

Refining involves the removal of nonglyceride compounds such as proteins, waxes, and free fatty acids. Phosphatides must be removed, as they will react with moisture and precipitate out. The type of refining referred to in this work is alkali refining, which involves the formation of soapstock by reacting free fatty acids with caustic soda (NaOH). Care must be taken so that glycerides do not react with the soda, which results in increased refining loss. This soapstock is then removed as a by-product of the refining process, and the result is a more neutralized oil, which increases oxidative stability (Johnson 2008).

Bleaching is performed to remove naturally occurring pigments, such as chlorophyll. This is not only done for better consumer acceptance, but chlorophyll is also a prooxidant in the presence of light, so bleaching also is done to improve oxidative

stability. The bleached oil then proceeds to the winterization step. Winterization is a chilling step in which compounds with higher molecular weights precipitate and are filtered out. Deodorization follows, which is primarily for the removal of small volatile compounds such as aldehydes and ketones. These volatiles give undesirable flavors and off-odors to the oil. Peroxides are also removed during this step- they are known as powerful prooxidants during the chain reaction of autoxidation (Johnson 2008). The DHA oil used in this study was extracted from an algal source via fermentation in large drums. The fatty acid composition of the oil can be seen in Table 2 (Frankel and others 2002).

Table 2: Approximate fatty acid composition of commercially available RBD DHA oil

Fatty acid	%
C12:0	4.4
C14:0	15.6
C16:0	12.8
C16:1 n-7	1.4
C18:0	0.9
C18:1 n-9	21.6
C18:2 n-6	0.8
C22:6 n-3	42.4
Other	0.2

The objective of this work was to study the effects of processing on the oxidative stability of algal DHA oil. Many studies have been performed to investigate processing effects on soybean oil; however no published work exists at this time on the effects of processing steps on DHA oil.

Materials and Methods

Crude, refined, bleached, winterized and deodorized oils from the same batch were provided by a commercial DHA oil supplier (Columbia, MD). An initial 1100 ppm

tocopherol was added during crude oil extraction, however no other additions of antioxidants were performed for research purposes. Oils were stored in the freezer throughout the experimental period to prevent oxidation and other changes in composition. Teflon coated rubber septa, aluminum caps, and serum bottles were purchased from Supelco, Inc. (Bellefonte, PA).

Two grams of each oil sample (refined, bleached, winterized, and deodorized oils) were transferred into 10-ml serum bottles which were tightly sealed with Teflon lined rubber septa and aluminum caps. All samples were prepared in triplicate and stored under light at 2500 lux at 25°C for 5 days. Statistical analysis was performed using Microsoft Excel 2007 software.

For the determination of headspace oxygen depletion, 100 µL of headspace was extracted from each sealed bottle and injected into the gas chromatograph (Hewlett-Packard 5880A) using a thermal conductivity detector. Molecular oxygen content of the headspace was determined with a 6 ft x 1/8 inch stainless steel column packed with Molecular Sieve 13x and a Nitrogen flow rate of 20 ml/min. Temperatures of the oven, injection port, and detector were 35, 200, and 250 °C, respectively. Sensitivity of the machine was determined by injecting l00 µL atmospheric air into the gas chromatograph and reading the oxygen peak area before each sample. Oxygen content was expressed as electronic counts of peak area of oxygen. All samples were analyzed in triplicate.

For the determination of headspace volatile compounds, solid phase microextraction (SPME) was used to collect volatiles from the headspace of the sample bottles. A polydimethyl silane- divinyl benzene fiber was used for maximum adsorption of the compounds. The fiber was placed in the injection port of the gas chromatograph

(Hewlett-Packard 6890 Series) for 2 minutes to allow all volatiles to cook off the fiber onto the column. Total volatile content was determined using a Supelco SP 2380 fused silica capillary column (100mm x 0.25 mm x 0.3μm) with an N_2 carrier gas (24ml/min) with a 30-min run time and a flame ionization detector. Temperatures of the oven, injection port, and detector were 50, 250, and 250 ºc, respectively.

Results and Discussion

Headspace Oxygen Analysis

The effects of processing steps on the headspace oxygen depletion of DHA oil are shown in Figure 2. As expected, oxygen content in the headspace decreased over storage time from 0 to 5 days. This occurs due to the reaction between oxygen in the headspace and DHA in the oil. When the oxygen reacts with the DHA, hydroperoxides and peroxy radicals are formed, which are direct products of lipid oxidation, making headspace oxygen depletion a reliable measurement of lipid oxidation. The oxygen content in the refined DHA oil decreased from 20.9% on Day 0 to 6.7% on Day 5, showing the most headspace oxygen depletion and therefore the lowest oxidative stability of the 4 oils studied. Bleached, winterized, and deodorized oils had headspace oxygen depletions of 20.9% on Day 0 to 8.3%, 7.8% and 7.8%, respectively. The bleached, winterized, and deodorized oils were determined not to be statistically different from each other ($p < 0.05$), and indeed the final headspace oxygen percentages were very similar. It can be seen in Figure 2 that the second phase of DHA oil processing, the bleaching step, had an important role in increasing the oxidative stability of the DHA oil when noting the difference in the amount of headspace oxygen depletion between refined and bleached oil. The two both started at 20.9%; however the rate of depletion for refined oil was

much greater (again ending at 6.7%), showing that refined oil had lower oxidative stability than bleached oil (ending at 8.3%). The removal of photosensitizers such as chlorophyll along with the removal of metal ions in the bleaching step is most likely responsible for the increased oxidative stability of bleached oil as compared to refined oil.

Focusing on the refined oil sample, a steeper slope can be seen on days 1-2, coinciding with a higher rate of headspace oxygen depletion, with the rate slowing slightly on days 3-5. This may be due to a decrease in the concentration of oxygen in the headspace, thereby causing a decrease in the reaction rate. A similar trend can be seen in the bleached, winterized and deodorized samples.

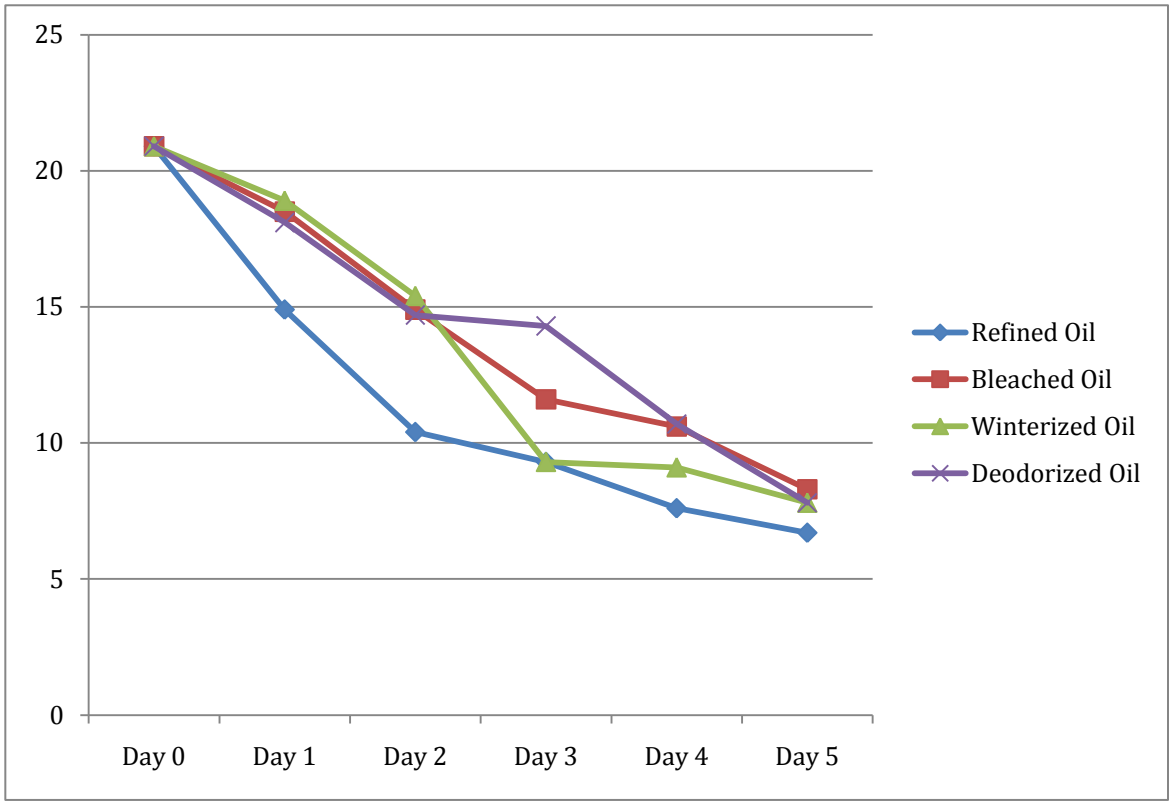

Figure 2: Effects of oil processing steps on the headspace oxygen depletion of DHA oil during storage under light at 25 C

Total Volatile Content Analysis

The effects of processing on the total volatile compound formation during storage under light at 25°C can be seen in Figure 3. These volatile compounds are formed via the lipid oxidation reaction between oxygen and DHA in the oil. Hydroperoxides are formed and then broken down into smaller compounds known as volatiles, for example aldehydes and ketones, which are responsible for the off-flavors and rancid odors associated with oil that has been oxidized. Flavor and odor quality of oil is directly related to volatile compound formation- the more volatiles formed, the more rancidity is observed. Certain volatile compounds have been associated with rancid flavors, such as 2, 4 decadienal in soybean oil (Min 1981) and 2, 4 heptadienal in fish oils such as DHA (Karahadian and others 1985). As expected, the total volatile content in DHA oil increased over storage time from Day 0 to Day 5. In refined oil at Day 0, the volatile content was already much higher than the other oils. Volatile content for refined oil at Day 0 was approximately 2,770,500 electronic counts, increasing to approximately 4,325,900 electronic counts on Day 5 for an average of approximately 4,041,000 electronic counts (ec). Refined oil showed the most volatile compound formation and was again determined to have the least oxidative stability of the oils tested. Not only did refined oil start out with the highest volatile content, the rate of volatile compound formation was much higher than that of the other oils. Similar to the trend observed in headspace oxygen depletion, the rate of volatile compound formation was higher on days 1-3, with a decrease in the rate of formation on days 4 and 5. On these days, the decrease can be explained by the decomposition and dimerization of volatile compounds formed previously into non-volatile compounds not detected in the gas chromatogram. An

example of this type of reaction is the formation of a hemiacetal via the reaction between an aldehyde and an alcohol. This same type of trend was not observed in the volatile compound formation in bleached, winterized, and deodorized oils, and the overall formation and rate of formation of volatile compounds in these three oils was much lower than in refined oil.

Bleached, winterized, and deodorized oils showed averages of approximately 482,100 ec, 437,200 ec, and 405,800 ec, respectively. As with the headspace oxygen depletion, the bleached, winterized, and deodorized oils were found to not be statistically different from each other ($p < 0.05$). Figure 3 shows that bleaching proved to be very important in the removal of volatile compounds when compared with refined oil at Day 0. As with headspace oxygen depletion, the removal of photosensitizers such as chlorophyll and metal ions during bleaching significantly increased the oxidative stability of the DHA oil. Winterizing and deodorizing removed the remaining volatile compounds at Day 0 as can been seen in deodorized oil, which started with 0 ec volatile content. The small decrease at Day 0 in winterized oil as compared to bleached oil, while not statistically significant, is interesting, and can most likely be attributed to the filtration step used to remove the precipitated higher melting point compounds which winterized is designed to eliminate. Some volatiles may also be filtered at this step.

Bleached and winterized oils remained relatively constant in their volatile content during storage; however deodorized oil showed a trend upward, especially at Days 4 and 5. This is believed to be the result of the removal of tocopherols and other natural antioxidants during the deodorizing process that occurs along with the removal of volatile compounds. The removal of these antioxidant compounds may be causing the

deodorized oil to begin losing oxidative stability over time during storage (Tomaino and others 2005).

The correlation coefficients (r) for refined, bleached, winterized, and deodorized oils, r-values were calculated as: -0.92, -0.98, -0.95, and -0.98, respectively. High correlations between these data sets indicate that oxygen was depleted in the headspace as it reacted with DHA to form volatile compounds and hydroperoxides in a gas tight sample vial. These results show that the combination of headspace oxygen depletion and total volatile compound formation can be used as a reliable analytical method for the measurement of lipid oxidation.

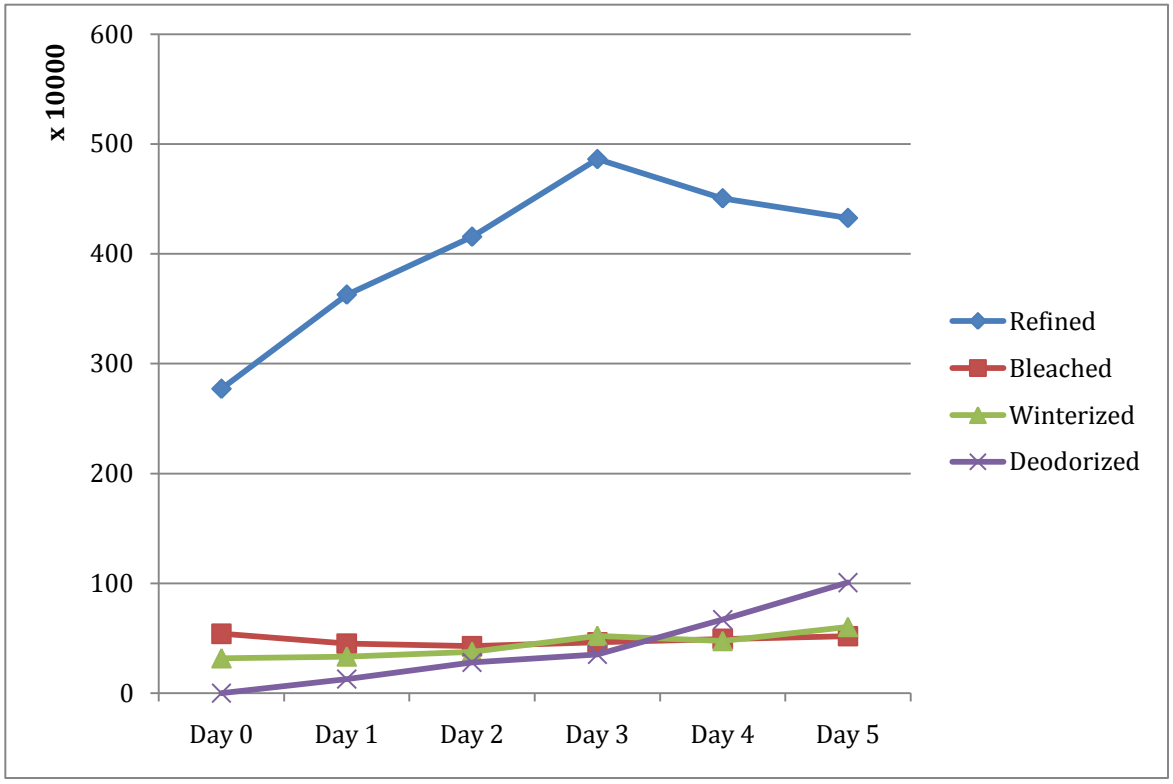

Figure 3: Effects of processing on the total volatile content of DHA oil during storage under light at 25 C

Conclusion

Bleaching proved to be the crucial processing step for increasing the oxidative stability of DHA, most likely due to the removal of prooxidant compound such as chlorophyll and metal ions. Winterizing and deodorizing further removed volatile compounds, and deodorized oil showed an initial volatile content of 0 electronic counts. Deodorizing, however, also partially removed natural antioxidants present in the oil, the effects of which can be seen by the higher increase in the rate of volatile compound formation during storage in the deodorized oil as compared with the bleached and winterized oils.

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